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EXTRACORPOREAL PERFUSION WITHOUT ANTICOAGULATION AND THE RESPON--ETC(U)  
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TECHNICAL REPORT NO. 125

EXTRACORPOREAL PERFUSION WITHOUT ANTICOAGULATION  
AND THE RESPONSE TO ENDOTOXIN

B. K. Beller, L. T. Archer,  
S. D. Kosanke, and L. B. Hinshaw

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Prepared for Publication  
in  
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Biology and Medicine

University of Oklahoma Health Sciences Center  
Departments of Physiology & Biophysics, Surgery, and Pathology  
Oklahoma City, Oklahoma

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SCHEMATIC DIAGRAM OF PERFUSION SYSTEMS

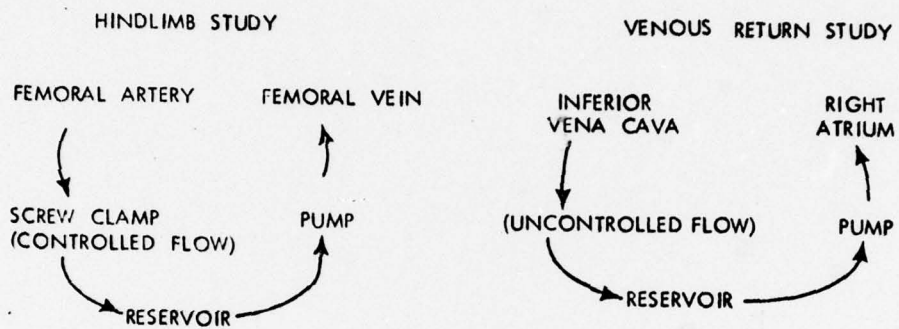


FIGURE 1

femoral artery of each dog was cannulated with a flexible plastic catheter advanced into the distal aorta and was used to monitor mean systemic arterial pressure (MSAP) and heart rate (HR) and to sample blood for hematocrit (Hct), blood glucose and clotting time determinations. The left femoral vein was cannulated in six of twelve dogs to be available for the endotoxin infusion. Large-bore plastic catheters filled with saline were connected at one end to the right femoral artery of the animal so that blood would flow via a screw clamp to control the flow into a saline-filled 200-ml plastic reservoir and be returned via a roller-type pump to the right femoral vein. No animal received an anticoagulant of any kind. Rectal temperature was recorded and blood flow was measured via a calibrated pump.

Six of twelve animals received a 2-hour infusion of LD<sub>100</sub> E. coli endotoxin (B5 strain; Difco, Detroit, Michigan), 2 mg/kg (1 mg/ml), beginning 15 minutes after establishment of perfusion. Sample times were every 15 minutes the first hour and every 30 minutes thereafter through 6 hours. At the end of the experiments, all twelve animals were sacrificed with additional sodium pentobarbital and representative samples of heart, lung, liver, spleen and kidney were fixed in neutral buffered 10% formalin. Paraffin-embedded tissue, sectioned 4-6 microns, was stained with hematoxylin and eosin and Mallory's phosphotungstic acid hematoxylin and examined by light microscopy.

(2) "*Venous return*" study. Twelve dogs weighing  $13.5 \pm 2.8$  kg were intravenously anesthetized with sodium pentobarbital, 30 mg/kg body weight, and the right and left femoral arteries of each animal were cannulated. The right femoral artery was then available to sample blood for Hct, pH,

glucose, clotting time, fibrinogen and platelets. MSAP and HR were monitored via the left femoral artery. Each of the six animals chosen to receive a one-hour infusion of LD<sub>100</sub> E. coli endotoxin 15 minutes after establishment of perfusion had its right femoral vein cannulated also.

Each dog was connected to a constant volume Starling respirator with 2-3 inches of positive end expiratory pressure. A mid-line thoracotomy was then performed and the right atrium was cannulated with saline-filled wide-bore plastic tubing such that blood could be delivered into the right heart at a controlled flow rate via a roller-type pump. Next, the inferior vena cava was quickly cannulated with a saline-filled cannula, its tip placed downstream from the confluence of the hepatic vein and inferior vena cava. The distal tip of the cannula was lowered sufficiently to maintain orifice inferior vena cava pressure at or below atmospheric pressure. Venous outflow drained passively into a saline-filled 300-ml plastic reservoir surrounded by a water bath maintained at 40°-42°C, and returned to the right atrium via a roller-type pump, adjusted to maintain the volume of the blood in the reservoir at a constant value during the 4 hours of perfusion. Under these conditions, venous return and cardiac inflow were equal.

No animal received an anticoagulant of any kind. Sample times were every 15 minutes for 4 hours. Blood flow was measured via a calibrated pump. MSAP and HR were monitored on a Sanborn recorder. Rectal temperatures were obtained using a Tele-Thermometer probe. Blood glucose concentrations were determined using a Beckman glucose analyzer, and an Instrumentation Laboratories blood gas analyzer was used to determine pH.



Clotting time was ascertained at room temperature by a modified Lee-White procedure (10). Platelet and fibrinogen determinations were conducted as previously described (11,12). Experiments were terminated by giving each animal an overdose of sodium pentobarbital, and tissue specimens of heart, lung, liver, kidney and spleen were fixed, prepared, sectioned, stained and examined by light microscopy as in the hindlimb study.

Data from both preparations were analyzed using the student "t" test. Only values of less than 0.05 were considered statistically significant.

## RESULTS

(1) *Hindlimb study.* The mean ( $\pm$ SE) mean systemic arterial pressure (MSAP), blood glucose, hematocrit (Hct), clotting time, and heart rate (HR) are shown in Figures 2A and 2B for control and endotoxin-treated groups during the 6-hour perfusion period. Control dogs demonstrated no significant changes from zero time in MSAP, blood glucose or Hct during the 6-hour period. However, in the endotoxin-treated group, MSAP significantly decreased starting at 90 minutes, blood glucose decreased from 240 minutes, and Hct increased from 60 minutes to the end of the experiment ( $p < 0.02$ ).

Clotting time increased significantly in both groups from 30 minutes to termination of the studies ( $p < 0.02$ ). The mean zero time clotting time value for both groups was 10 minutes. Control animals reached a maximum of 15 minutes by 150 minutes of perfusion and remained relatively constant. Endotoxin-treated animals had a progressive rise to a maximum clotting time of 19 minutes at 6 hours. The mean initial HR for the controls was  $179 \pm 13/\text{min}$  while in the endotoxin group it was  $138 \pm 8/\text{min}$ ; consequently,

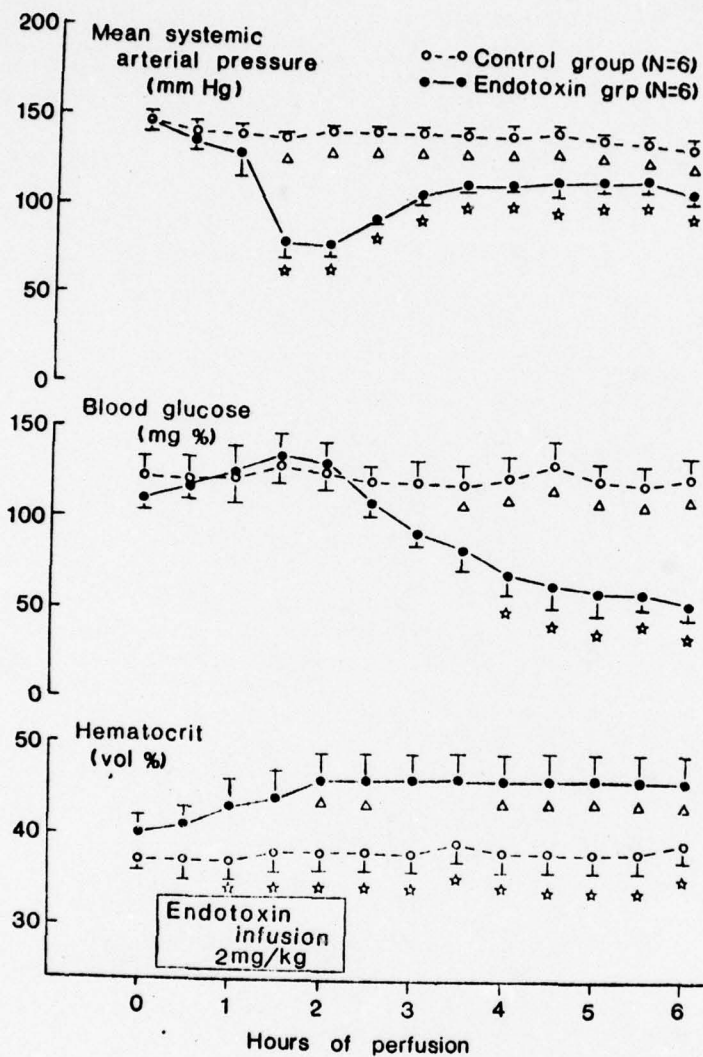


FIGURE 2A. Hindlimb study. Mean ( $\pm$ SE) MSAP, blood glucose, and Hct parameters during six hours of extracorporeal perfusion without anticoagulation.  
 X = control group, each dog compared to its zero time value,  $p < 0.02$ .  
 \* = endotoxin group, each dog compared to its zero time value,  $p < 0.02$ .  
 Δ = endotoxin group compared to control group,  $p < 0.05$ .

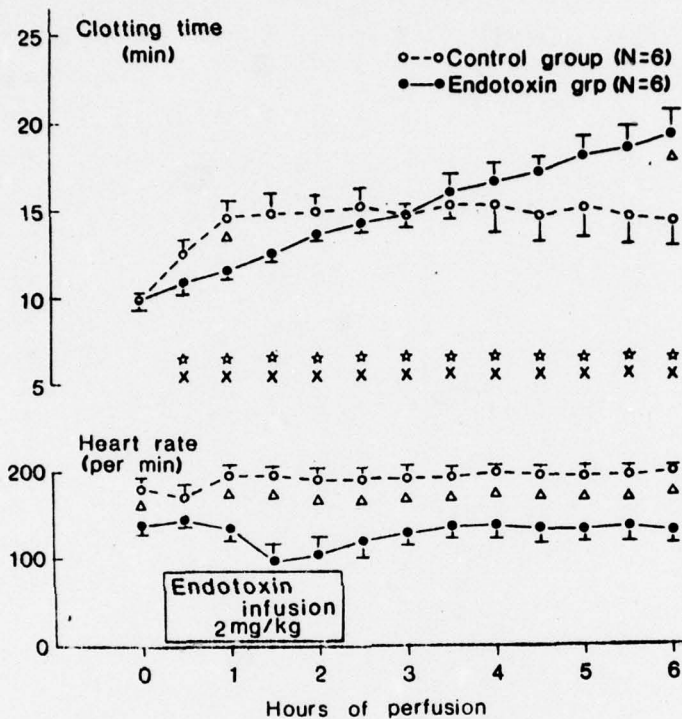


FIGURE 2B. Hindlimb study. Mean (+SE) whole blood clotting time and HR parameters during six hours of extracorporeal perfusion without anticoagulation. See Figure 2A for symbols of significance.



the two groups were significantly different from the beginning. However, neither group demonstrated a significant difference from its own control at any time.

The mean rectal temperature progressively increased in the control group from 90 minutes and in the endotoxin group from 150 minutes, with both groups ending the study at 39.3°C ( $p < 0.03$ ). Blood flow was maintained relatively constant in each experiment by adjusting the screw clamp on the arterial outflow tubing. Blood flow of the control group averaged  $408 \pm 63$  ml/min or  $19 \pm 2$  ml/kg/min; that of the endotoxin group was  $587 \pm 3$  ml/min or  $25 \pm 2$  ml/kg/min.

Light microscopy revealed no fibrin thrombi deposition in the heart, lung, liver, spleen or kidney of any of the twelve animals. Lesions observed in the six endotoxin-treated dogs but not in the controls were increased numbers of segmented neutrophils in the portal veins, central veins, sinusoids, and periportal spaces of the liver; moderate to severe congestion, edema and hemorrhage of the liver; and excess numbers of segmented neutrophils in the capillaries and small veins of the lungs.

(2) "*Venous return*" study (Figure 3). The MSAP of the control group in the venous return study at 15 minutes of perfusion was  $106 \pm 9$  mmHg and progressively rose to  $136 \pm 8$  mmHg in 4 hours. MSAP significantly decreased in the endotoxin-treated dogs compared to the control animals from 45 minutes until termination of the study ( $p < 0.02$ ) and simultaneously decreased from its own initial perfusion value of 116 mmHg to a maximum low of 55 mmHg at 120 minutes ( $p < 0.005$ ). Heart rates remained relatively constant in both groups compared to their initial perfusion values; however, the groups were significantly different from each other between 90 and 210 minutes.

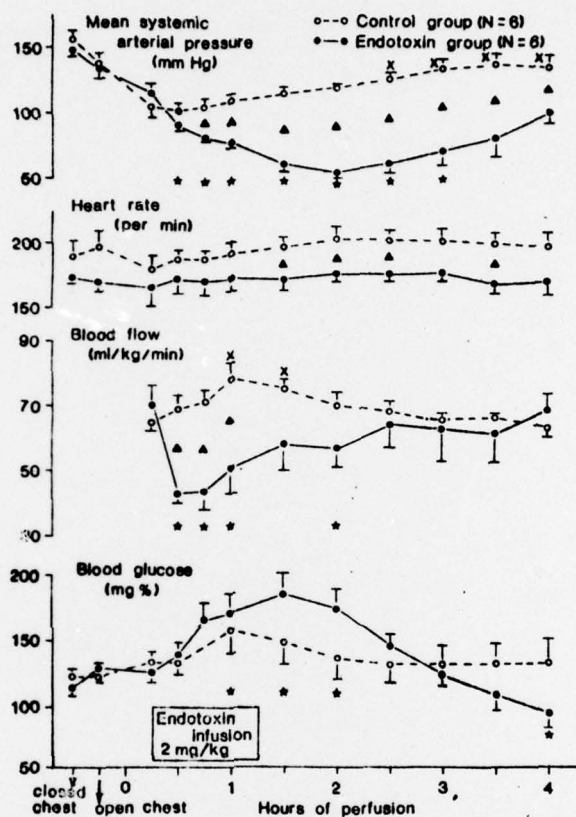


FIGURE 3. Venous return study. Mean ( $\pm$ SE) MSAP, HR, blood flow, and blood glucose parameters before and after thorotomY and during four hours of extracorporeal perfusion without anticoagulation.

X = control group, each dog compared to its 15 minute perfusion value,  $p < 0.05$ .

\* = endotoxin group, each dog compared to its 15 minute perfusion value,  $p < 0.05$ .

$\Delta$  = endotoxin group compared to control group,  $p < 0.05$ .

Mean blood flow in the control group increased at 60 and 90 minutes ( $p < 0.05$ ), but otherwise remained at control perfusion levels ( $p > 0.05$ ). Blood flow decreased in the endotoxin-treated animals during endotoxin administration (from 30 to 60 minutes,  $p < 0.02$ ), but essentially recovered to that of the controls by 90 minutes ( $p > 0.05$ ). Blood glucose values remained relatively constant in the controls during the 4-hour period ( $p > 0.05$ ). When blood glucose levels were compared between groups, there were no significant differences at any given time, although the endotoxin group did demonstrate higher mean glucose levels from 60 through 120 minutes compared to its initial perfusion level ( $p < 0.05$ ).

Rectal temperatures of the controls remained relatively constant ( $37.5^{\circ}$  to  $38.1^{\circ}\text{C}$ ,  $p > 0.05$ ), but they decreased in the endotoxin group from 30 minutes ( $37.9^{\circ}$  to  $37.1^{\circ}\text{C}$ ,  $p < 0.05$ ) throughout the study. The mean hematocrits of the controls decreased from their own 15-minute perfusion time value from 2 through 4 hours ( $p < 0.05$ ) but were not significantly different from those of the endotoxin group. Mean pH values remained relatively constant in the control group. The pH of the endotoxin-treated animals was significantly lower than that of the controls and of their own initial perfusion pH from 90 through 240 minutes. Averaged mean pH values during that time were  $7.29 \pm 0.01$  for the endotoxin group and  $7.42 \pm 0.01$  for the control dogs.

Both groups reached infinite clotting times ( $> 18$  hours) after approximately 60 minutes of perfusion, and the individual values are shown in Table I.

Table II shows mean platelet and fibrinogen concentrations before and after mid-line thoracotomy and following 60 and 120 minutes of perfusion. The only significant difference occurred at 60 minutes of perfusion when the endotoxin-treated group's platelets fell markedly.



TABLE I. Effect of Venous Return Procedure and Endotoxin on Clotting Time

		Time of Perfusion (min)									
Clotting Time (min)*		Closed chest	Open chest	+15	+30	+45	+60	+90	+120	+150	
Control Group	1	14	14	15	17		19	22	23	∞**	
	2	13	14	14	18	24	∞				
	3	11	12	18	26	∞					
	4	9	10	19	29	34	∞				
	5	16	16	28	31	∞					
	6	13	14	17	29	∞					
Mean(±SE)		13(1)	13(1)	19(2)	25(3)						
Endotoxin-Treated Group	1	10	12		13	14	22	∞			
	2	12	12		18	∞					
	3	14	16		18	27	∞				
	4	13	13		26	∞					
	5	13	14		31	∞					
	6	16	18		35	∞					
Mean(±SE)		13(1)	14(1)		24(4)						

\*Lee and White (modified)--arterial blood

\*\*>18 hours

TABLE II. Effect of Venous Return Procedure and Endotoxin on Platelet Count and Fibrinogen Level

Platelets/mm <sup>3</sup> *		Closed chest	Open chest	Minutes of Perfusion	
				+60	+120
Control group	Mean	371,800	338,700	232,200	179,200
	SE	48,400	45,200	59,300	43,100
	N	5	6	5	6
Endotoxin-treated group	Mean	323,300	292,500	63,800	107,000
	SE	76,300	16,200	18,500	43,800
	N	6	6	6	6
Fibrinogen (mg/dl)*					
Control group	Mean	246	186	128	131
	SE	61	12	23	31
	N	4	4	4	4
Endotoxin-treated group	Mean	293	244	129	83
	SE	66	71	28	22
	N	6	5	6	6

\*Arterial blood

Light microscopy revealed no fibrin thrombi in the heart, liver, spleen or kidney of any of the twelve dogs. However, a few small, loosely-arranged fibrin thrombi were present in the pulmonary vessels of two of the six endotoxin-treated dogs. Other lesions observed in the six endotoxin-treated dogs but not the controls were increased numbers of segmented neutrophils in the portal veins, central veins, sinusoids and periportal spaces of the liver, and excess numbers of segmented neutrophils in the capillaries and small veins of the lung. In one of the six endotoxin-treated dogs moderate to severe centrilobular hemorrhages of the liver were observed.

#### DISCUSSION

Findings from this study show that extracorporeal perfusion in dogs can be successfully carried out in the absence of exogenously administered anticoagulant.

The "hindlimb" perfusion study consisted of an arteriovenous shunt, in contrast to Fletcher and others' venovenous procedure (8,9) and the present venous return study. Lower control blood flows were obtained in the hindlimb study than in the venous return study by controlling the arterial outflow. The control group's whole blood clotting times leveled off early and were limited to less than 30 minutes. Hemodynamic and metabolic parameters of the control dogs remained relatively constant and no adverse tissue changes were noted.

Results from the "venous return" experiments are in close agreement with those obtained by Fletcher and others (8,9) in dogs and baboons except for clotting times, which ultimately exceeded 18 hours in our study. The



controls in the present studies were very stable. This is demonstrated by the relatively constancy of the mean systemic arterial pressure, heart rate, hematocrit, pH, blood flow, and blood glucose during 4 hours of perfusion, and the absence of fibrin thrombi and adverse cellular changes in the tissues of the animals at the end of the experiment. This was not the case in venous return studies previously performed in this laboratory using exogenous anticoagulation (heparin) (5,6). In those experiments, controls became unstable after approximately 75 minutes of perfusion, resulting in excessive bleeding, systemic hypotension, and decreased cardiac output. Data, therefore, suggest that an unheparinized perfusion system would be an improved model.

The mechanisms resulting in increased whole blood clotting times in the hindlimb perfusion study and the infinite clotting times in the venous return perfusion experiment are not apparent. Secondary fibrinolysis is associated with decreased fibrinogen and platelet levels (13,14). Perhaps the decrease in platelets and fibrinogen observed in the present study reflect stimulation of the coagulation system with secondary fibrinolysis, particularly in the venous return experiment. The fact that fibrin deposition was not observed in the tissues of the perfused animals and the fact that Fletcher and others (8,9) demonstrated elevated levels of fibrin split products in their extracorporeal membrane oxygenation study underscores this probability.

Although endotoxin shock has long been associated with altered or abnormal clotting mechanisms (15,16), previously reported animal perfusion studies in endotoxin shock have been conducted using anticoagulation (5-7). Following successful establishment of the two perfusion systems in the

present study using no anticoagulant, the system's response to an infusion of LD<sub>100</sub> E. coli endotoxin was observed. Typical endotoxin responses were clearly evident in the initial phase of shock; i.e., decreased blood pressure and flow. In the latter phase, certain improvements over the endotoxin perfusion studies (5-7) were noted: blood pressure remained at higher levels in the hindlimb experiments and mean blood flow and pressure returned to control values in the venous return study. The clotting times of the endotoxin group increased more than those of the controls during the latter phase of the hindlimb study, suggesting that anticoagulation factors may have been induced by both extracorporeal perfusion and by endotoxin. It is hypothesized that the improvements of blood flow and pressure which correlated with the increased clotting time values may bear a relationship to increased fibrinolytic activity.

#### SUMMARY

This study shows that an extracorporeal perfusion system without anticoagulation can be established in the dog under certain conditions. It also points to a model to study the effects of endotoxin without heparin interference and as another model for studying the fibrinolytic system.

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The present study was designed and carried out in an attempt to establish an extracorporeal perfusion system without anticoagulation and to study how this system affects the animal's response to endotoxin. The results of the study show that an extracorporeal perfusion system without anticoagulation can be established in the dog under certain conditions. It also points to a model to study the effects of endotoxin without heparin interference and as another model for studying the fibrinolytic system. ↙

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